350-Pos
Activation of a Helicase Motor Upon Encounter With a Specific Sequence in the DNA Track
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DNA helicases are ATP-driven motor proteins that catalyse the separation of duplex DNA into its component single-strands. These are key intermediates in many cellular DNA transactions including replication, recombination and repair. In this study, we have investigated the kinetics of DNA translocation and unwinding by the AddAB helicase-nuclease; an enzyme which is involved in the initiation of double-stranded DNA break repair by homologous recombination. We show that AddAB displays extremely rapid and processive DNA unwinding activity powered by a single Superfamily 1A helicase domain. Uniquely, the helicase activity of AddAB is dramatically stimulated upon encounter in cis with a specific regulatory sequence (Chi) embedded in the DNA track. The molecular basis for this activation of helicase activity and its implications for our understanding of helicase mechanisms in general will be discussed.

351-Pos
A Closer Look At the Unwinding Initiation By Twinkle-The Human Mitochondrial DNA Helicase
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TWINKLE (T7gene4 protein like protein with intra-mitochondrial nucleoid localization) is a hexameric helicase involved in the maintenance of the mitochondrial genome in mammalian cells. It is encoded by PEO1 gene of chromosome 10. Mutations in PEO1 have been linked to mitochondrial DNA (mtDNA) deletions, which lead to various neuromuscular disorders. TWINKLE shares about 46% amino acid sequence similarity with T7gene4 protein (gp4), the bifunctional primase-helicase of bacteriophage T7. Even though T7gp4 is one of the best studied helicases, studies on TWINKLE are only in its nascent stage. In this study, we have purified TWINKLE to near homogeneity and have used it to study the kinetics of dsDNA unwinding by this helicase. We have focused on finding the optimal NTP and DNA substrate for unwinding by TWINKLE. Our studies show that UTP is the preferred substrate for fork DNA unwinding by TWINKLE in vitro. Fork DNA unwinding assays with a variety of 3′-tails indicate that the morphology of 3′-tail is crucial for unwinding by TWINKLE. This is a step towards understanding the mechanism of initiation of replication from the D-loop in the mitochondrial DNA. Our ultimate goal is to understand the mechanism of replication of mitochondrial DNA in vitro which in turn would enable us to define the molecular basis of various diseases associated with mutations in TWINKLE.

352-Pos
Stepping Mechanism of Bacteriophage T7 Helicase and Priming Loop Observed Using Single-Molecule Fret Methods
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Bacteriophage T7 gp4, a hexameric ring-shaped helicase, serves as a model protein for replicative helicases. T7 helicase couples dTTP hydrolysis to directional movement and DNA strand separation. Previous studies have shown that its DNA unwinding rate and dTTPase rate are sensitive to base pair (bp) stability.We confirm that the unwinding rate of T7 helicase decreases with increasing base pair (bp) stability. For duplexes containing $>$ 35% GC basepairs, we observed stochastic pauses during unwinding. Using an AT block followed by GC block as a calibration tool, we determine that the pausing occurs every 2-4 bp, regardless of detailed sequence composition. The dwells on each pause were distributed non-exponentially, consistent with several rounds of dTTP hydrolysis events before each unwinding step.
We also study the dynamics of the T7 replisome. We show that in the presence of T7 DNA polymerase, the primase does not slow down or pause the T7 helicase during primer synthesis. Furthermore, we observe a formation and disappearance of a ssDNA loop between the helicase and the primase domains during the unwinding/leading strand synthesis. Our results suggest a model whereby the priming loop allows the coordination of leading and lagging strand DNA synthesis by keeping the primer in physical proximity to the replication complex by assuring efficient primer utilization and hand-off to the polymerase.

353-Pos
Direct Observation of Twisting Steps During Rad51 Polymerization on DNA
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The human recombinase hRad51 is a key protein for the maintenance of genome integrity and for cancer development. This protein plays a central role is the DNA strand exchange occurring during homologous recombination. Here we report the polymerization and depolymerization of hRad51 on duplex DNA observed with a new generation of magnetic tweezers, allowing the measurement of DNA twist with a resolution of 5° in real time. At odds with earlier claims, we show that, after initial deposition of a multimeric nucleus, nucleoprotein filament growth occurs by addition of single proteins, involving DNA twisting steps of $65 \pm 5°$. Simple numerical simulations support that this mechanism is an efficient way to minimize nucleoprotein filament defects. This behavior, consisting of different stoichiometry for nucleation and growth phases, may be instrumental in vivo. Fast growth would permit efficient continuation of strand exchange by Rad51 alone while the limited nucleation would require additional proteins such as Rad52, thus keeping this initiation step under the strict control of regulatory pathways. Besides, our results combined with earlier structural information, suggest that DNA is somewhat less extended (4.5 versus 5.1 Å per bp) than by RecA, and confirm a stoichiometry of 3-4 bp per protein in the hRad51-dsDNA nucleoprotein filament.

354-Pos
Real-Time Visualization of Assembly and Disassembly of S. Cerevisiae Rad51 on Duplex DNA
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DNA double-strand break (DSB) is a lethal type of DNA damage. Homologous recombination is the most accurate method to repair broken DNA. The process of recombinational DNA repair consists of three phases. First, the ends of broken DNA are processed to generate a 3′-ended single-strand DNA (ssDNA) tail on which the DNA strand exchange protein self-assembles. Second, the protein-DNA complex searches for homology on a donor double-strand DNA (dsDNA), and catalyzes the pairing and exchange of DNA strands. Finally, the heteroduplex DNA product is resolved.
In S. cerevisiae, DNA strand exchange is catalyzed by Rad51 protein. Rad51 forms a right-handed helical nucleoprotein filament in an ATP-dependent manner. In the filament, Rad51 occupies 3-4 nucleotides and stretches DNA to ~150% of its B-form length. Interestingly, Rad51 binds to dsDNA with a similar affinity as for ssDNA, even though the functional form of Rad51 requires assembly on ssDNA. Also, since Rad51 filament formation on dsDNA inhibits DNA strand exchange, the turnover of Rad51 from dsDNA is stimulated by a dsDNA translocase, Rad54.
We used single-molecule fluorescence microscopy to visualize both the kinetics of assembly and disassembly of Rad51 nucleoprotein filaments. Visualization was achieved either by directly imaging filament formation using fluorescent Rad51 protein, or by measuring the DNA length change due to Rad51 binding by fluorescently tagging the DNA end. We show that the nucleation of Rad51 on dsDNA requires about 3 monomers; that Rad51 assembly occurs through frequent nucleation; and that the Rad51 filament is stabilized by maintaining ATP-bound state.

355-Pos
Coupling DNA Unwinding Activity With Primer Synthesis in the Bacteriophage T4 Primosome
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The unwinding and priming activities of the bacteriophage T4 primosome, which consists of a hexameric helicase (gp4) translocating 5′ to 3′ and an oligomeric primase (gp6) synthesizing primers 5′ to 3′, have been investigated on DNA hairpins manipulated by a magnetic trap. We find that the T4 primosome continuously unwinds the DNA duplex while allowing for primer synthesis through a primosome disassembly mechanism or a new DNA looping mechanism. A fused gp6-gp4primosome unwinds and primes DNA exclusively via the DNA looping mechanism. Other proteins within the replisome control the partitioning of these two mechanisms, which disfavor

primosome disassembly, thereby increasing primase processivity. In contrast, priming in bacteriophage T7 involves discrete pausing of the primosome, and in Escherichia coli it appears to be associated primarily with dissociation of the primase from the helicase. Thus nature appears to use several strategies to couple the disparate helicase and primase activities within primosomes.

356-Pos

Single Molecule Study on Incorporation Efficiency of DPO4 and Klenow Fragment in the Presence of BPDE Adduct

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It's well known that the binding of DNA adducts such as benzo[a]pyrene-diol-epoxide (BPDE) to DNA template strand can impede or block DNA synthesis during the process of DNA replication. While DNA synthesis involving high fidelity replicative A-family polymerases such as Klenow fragment are blocked by DNA adducts, members of Y-family DNA polymerases such as Sulfolobus solfataricus P2 DNA polymerase IV (DPO4) can bypass the DNA adducts and resume the DNA synthesis. Understanding the functional differences between A-family and Y-family DNA polymerases in the process of DNA replication and the mechanism of bypassing DNA adducts is of great value to explain the cause of mutagenesis. We introduce an assay by anchoring DNA molecules to the modified surface to study the incorporation efficiency of DPO4 and Klenow fragment with the presence of BPDE adduct at single molecule level. Specifically, we anchor fluorescent labeled DNA template onto this surface with the adduct site open for nucleotide incorporation, photobleach the labels and flow the polymerases and labeled nucleotides into the hybridization cell. Using Total Internal Reflection Fluorescence Microscopy (TIRFM) we identify the time sequence incorporation of the nucleotides onto the anchored DNA template by identifying the location of the labeled nucleotide from TIRF images. We further quantify the signal densities of the images obtained from the two different polymerases, thus examining whether incorporation reactions have been executed and quantifying the incorporation efficiency of the polymerases.

357-Pos

DNA Polymerization in Optical Tweezers

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Molecular motors associated with nucleic acids ensure replication, maintenance and expression of genetic information, which is crucial for life. Biochemical and biological studies have generated considerable knowledge about these molecular motors. However, these studies assess only sum reactions/effects due to ensemble averaging. Single molecule (SM) measurements overcome this shortcoming. Such measurements allow learning about individual motor functions, such as processivity, fidelity, step size, template dependence and generated forces. Transient intermediate states and rarely occurring events can also be observed.

We are developing an assay based on bacteriophage phi29 polymerase to study DNA/RNA polymerases in action using high resolution optical tweezers (OT) combined with fluorescence imaging (OT-SMF). We use an optically levitated 'dumbbell' assay: the nucleic acid (NA) construct features biotinylated and digixigeninated ends that tether two different kinds of microspheres (coated with streptavidin and anti-digoxigenin); the protein of interest is attached directly to the NA tether. Since in our double OT instrument one trap is stable whereas the other mobile, we can manipulate the tethers, detect changes in tether length and stiffness, apply different forces and simultaneously observe the fluorescently stained template/protein. We present preliminary results on DNA replication by phi29 polymerase.

358-Pos

Conformational Dynamics of Mismatch Recognition By E. coli Muts

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DNA mismatch repair protects the genome from spontaneous mutations by recognizing and repairing DNA synthesis errors in a pathway that is highly conserved. The MutS family of proteins initiate DNA mismatch repair by specifically binding mismatched or extrahelical bases and communicating the presence of damage to downstream repair proteins in an ATP-dependent manner. Previous structural studies have implied that MutS-induced conformational changes on DNA are central to damage recognition. Because the conformational changes occur on the timescale of seconds, it is difficult to obtain kinetic information on this highly dynamic process with traditional ensemble techniques. In this work, we use single molecule fluorescence resonance energy transfer (smFRET) to investigate the conformational dynamics of DNA in the presence of MutS from *E. coli*. FRET can measure changes in the relative distances between two fluorophores that are sufficiently close together (20-80 Å). In our experiments, DNA conformational dynamics are observed by detecting changes in the end to end distance of fluorescently labeled oligonucleotides with milliseconds time resolution. We present quantitative kinetic information on the rates of MutS binding and dissociation and the effect of nucleotides on the conformational dynamics of the mismatched DNA-protein complexes. Our results are discussed in the context of current models for DNA mismatch recognition.

359-Pos

Direct Visualization Of Joining of DNA Fragments By LigIIIβ Using Atomic Force Microscopy

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DNA ligaseIII is one of three mammalian DNA ligases (LigI, LigIII and Lig IV). LigIII is distinguished from the other ligases by the presence of a Zinc finger(ZnF) that improves ligation efficiency. Previously, it was demonstrated that LigIII has two different DNA binding modules (ZnF-DBD and NTase-OB) and a jack-knife model has been proposed to explain nick recognition and joining of double-strand breaks. However, the oligomeric state of LigIII in solution and during ligation, and the role of ZnF in end-end ligation are unknown. Using atomic force microscopy, we directly visualized a germ cell-specific form of DNA LigIII, LigIIIb and a delZnF mutant, their interactions with DNA and ligation products. We found no evidence for oligomerization of WT and delZnF LigIIIb in solution or when complexed to DNA. Importantly, WT and delZnF proteins exist in three distinct conformational states: closed, semi-extended, and extended conformations. While WTLigIIIb protein accesses all three conformational states significantly, delZnF LigIIIb occupies primarily the closed and semi-extended states, suggesting that ZnF is part of one wing as proposed in the jack-knife model. Furthermore, binding and ligation studies on nicked and non-nicked blunt-end DNA and DNA with 5′ overhang indicate that in addition to tandem joining of two linear DNA molecules, LigIIIb can mediate the ligation of a variety of higher order structures including three way junctions. In addition, with 5′ overhang DNA, compared to WT protein, delZnF promotes a small but significant occurrence of three-way junctions, lassoes and knots in the presence of MgCl2. These data suggest that monomeric DNA LigIIIb is capable of binding two DNA molecules simultaneously. Currently, we are testing the hypothesis that the ZnF in LigIIIb may be involved in quality control ensuring only tandem end-end ligation.

Transcription

360-Pos

Synergistic Action of RNA Polymerases in Overcoming the Nucleosomal Barrier

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During gene expression, RNA polymerase (RNAP) encounters a major barrier at a nucleosome and yet it must access the nucleosomal DNA. Various lines of in vivo evidence suggest that multiple RNAPs might increase transcription efficiency through nucleosomal DNA. Here we have quantitatively investigated this hypothesis by using E. coli RNAP as a model system and directly monitoring its location on the DNA via a single molecule DNA unzipping technique. When a single RNAP encountered a nucleosome, it paused with a distinctive 10-bp periodicity and was backtracked by an average distance of ~10-15 bp. When two RNAPs were elongating in close proximity, the trailing RNAP exerted an assisting force on the leading RNAP, reducing its backtracking and enhancing its transcription through a nucleosome ~5-fold. Taken together, our data indicate that histone-DNA interactions within a nucleosome dictate RNAP pausing behavior, and that alleviation of nucleosome-induced backtracking by multiple polymerases is a likely mechanism for overcoming the nucleosomal barrier in vivo.